Ligation

1. Use NEBioCalculator to establish a 5:1 molar:insert vector ratio with a vector DNA mass of 20ng.
2. Give a quick spin to the digestions and the ligase.
3. Place all the required reagents on ice.
4. Add 20ng (2µL) of destination plasmid digestion to a PCR tube.
5. Add the calculated volume of upstream part digestion and downstream part digestion to the reaction.
6. Add 2µL of 10X T4 DNA Ligase Buffer and 1µL of T4 DNA Ligase.
7. Bring volume up to 20µL with nuclease free water.
8. Incubate at room temperature overnight.
9. Heat inactivate at 80°C for 20 minutes.

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